Pre-Ph.D. Registration Synopsis

Molecular studies of soil bacterial isolates from some Ragi fields of Ranchi district

Submitted by

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2024

State of art:

Microbial communities play key roles in ecosystem processes by driving the Earth's biogeochemical rotations and diversity metrics related to the richness, evenness, and phylogenetic diversity of soil microbial communities. They can be used to analyze the functional consequences of variations in soil microbial diversity[1,2].

According to validated specialist reports, demands in agricultural production is predicted to increase by at least 70 % by 2050[3]. Such as an exponential growth in the human population (9.6 billion by the end of 2050) and depletion of non-renewable natural resources, the agricultural output is required to meet the rising world population's food needs under sustainable manners[4,5,6]. People are now becoming aware that intensive farming practices are fundamental to meet the future global agricultural demand[7]. That is why agricultural productivity is coming into focus worldwide and numerous research strategies are being pursued and added to address eco-friendly sustainability concerns, targeting to reduce non-renewable natural resources consumption and to eliminate the greenhouse gases emission due to their impact on climatic conditions[8,9]. Recommended eco-friendly approaches are available on the ability of microbial rhizospheric diversity for intensive vigorous crop production while preserving the biosphere. Accordingly, several approaches for better use of beneficial microbial resources are being proposed as low-input biotechnology to help sustain environmentally friendly agro-technological practices[10]. Deploying rhizospheric micro-organisms to enhance the intake of plant nutrients and to ameliorate the resistance against biotic and abiotic stress addresses a group of still little exploited reservoirs of opportunities in agriculture for sustainability[11,12,13,14].

However, the optimization of the plant-microbial relationship is an ambitious challenge defined by the complexity of interactions between plant and rhizospheric microbiomes as well as micro-organism to micro-organism and their reliance on efficient nutrient utilization and greater plant defense against the phyto-pathogens under diverse environmental conditions[15,16]. Since interaction between microbial communities and crops is governed by diverse ecological factors and agronomic management, the impact of adverse environmental factors must be considered, especially in the current climate change scenario, as they influence proper management of crop-microbiome interaction[17,14].

Problems to be investigated:

Soil of Ragi field is considered as one of the most diverse habitats on earth containing billions of bacteria as well as other organisms so in process of identification and selection throughput sequence techniques and the increasing number of culture library.

Objective of investigation:

1. Collection of the soil from Ragi fields of selected area, around Ranchi district.
2. Biochemical analysis of bacteria.
3. To study molecular bio typing techniques.
4. Analysis of phylogenetic trees.
5. Bio Statistical data analysis.

Motivation:

Association of bacteria with diversified Ragi crop field is important. We find out the symbiotic association of crop with bacteria. Ultimately the role is to reduce the use of fertilizers and that is beneficial for mankind. Biomagnification and pollution are the main problems of this area. So, if we are able to break the chain of biomagnification by using N_2 fixing bacteria, it will be of great importance for environment and mankind. Further on in the process of research, we may get other nitrogen fixing bacteria.

Hypothesis:

Investigation of the diversified bacteria of Ragi field's crop plants has soil fertility ability against certain crops. All bacteria are not beneficial for all crops because bacteria release some secondary metabolites in soil so in this place, field of research is very broad because in this place, we need a classification that which bacteria is beneficial or harmful for which crop. Because we have to work with metagenomics along with habitats of microbes.

The bootstrapping technique was employed as it is crucial for making well-informed evolutionary inter-pretations, as it addresses the inherent noise in genetic data and the complexities of evolutionary processes[18].

Research design and methodology:

1. **Isolation of Agricultural soil bacteria**

Bacteria will be isolated from different soil samples on Nutrient agar media. Sampling of soil from different agricultural fields will be done in areas located near Ranchi district of Jharkhand.

2. **Biochemical characterizations**

The isolated bacteria will be subjected to various biochemical techniques.

3. **Isolation of Genomic DNA**

Genomic DNA isolation will be done by Murmur's method[19], with slight modifications.

4. **Genomic characterizations- 16S rDNA Analysis**

Amplification of 16S rDNA using the universal Forward primers (5'-AGAGTTTGATCCTGGCTCAG-3') & Reverse Primer (5'-ACGGCTACCTTCTTAGCACTT-3')[20] will be done.

RFLP analysis

After that, Restriction Fragment Length Polymorphism (RFLP) analysis of 16S rDNA Product will be done using restriction enzymes.

RAPD analysis

Different sets of random primers will initially screen for their ability to amplify DNA of different strains. The reaction mixture will consist of dNTP's, oligonucleotide Primers, 10 mM Tris-HCL, pH 8.3, 50 mM KCI, 1.5 mM $MgCl_2$ and 1.25 units of *Taq* polymerase. Thermo cycler will be programmed for approx 35-40 cycles. After extraction with chloroform, the effectiveness of DNA amplification will be monitored by electrophoresis on 1.5 % agarose gel. The gels will be visualized under UV Trans-illuminator.

Phylogenetic analysis using bioinformatics tools

Clustering analysis of PCR products will be performed using bioinformatics tools. The bands that will obtain in gel can be tabulated in the form of matrix (0, 1) and then similarity will calculate with Jaccard coefficient using software for clustering of data which is based on un-weighted pair group method with arithmetic averaging (UPGMA).

Tools to be used:

- Petri-plates
- Conical flasks
- Beakers
- Test tubes
- Micropipettes
- PCR
- Gel documentation system
- Gel electrophoresis
- Cooling Centrifuge
- Laminar air flow
- Incubator
- Hot air oven
- Weigh balance
- pH meter
- Thermomixer
- Eppendorf tubes
- Micropipettes Tips
- Autoclave

Significance of study:

Microorganisms are known to be present in all biosphere regions. They add to improvement of environment in various ways. Presently the topic reveals the utility of microbial strains especially agricultural bacteria in relation to enrichment of soil by the process of molecular techniques. Genomic characterization will certainly help in classifying the population using DNA BARCODES. The technology offers advantages over biochemical characterization of microbial strains as combination and arrangement of bases in genome shows consistency there by more suitable identification of unseen microbes.

Its relevance to the present-day problems and need to society:

- Loss of soil fertility due to which productivity is also decreasing.

- Decrease in microbial populations due to dryness and drought.
- Environment is polluted by the use of chemical fertilizers, pesticides, and insecticides.
- Population is main cause of climate change and diverse effect on human's health
- Number of other organisms mutated or exited due to pollution

Contribution to the existing knowledge:

This involves creating an environment that allow these microorganisms to thrive and interact harmoniously with the plant. The goal is to develop a technique that can reliably enhance plant health and performance. One aspect of research in this area focuses on investigating the specific compositions of microbial communities in the soil. Different combinations of microorganisms can have varying effects on soil fertility and subsequently impact on the development of plants that grow in that soil. Through careful analysis, researchers seek to uncover the intricate relationships between these micro-organisms and the overall health of both the soil of Ragi fields and the plants.

Future work:

- In present era microbes are very important weapon to control pollution, in future we can go through the modification and use these microbes to digest the harmful chemical compounds
- If we understand the relationship between microbes and soil of Ragi crop then we can increase the productivity and control the pollution.
- Microbes also contribute in making the drugs.

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